

GENERAL PRINCIPLES

PRINCIPLES OF ANTIEPILEPTIC DRUG ACTION

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The ideal antiepileptic drug protects against seizures without adversely affecting the function of the central nervous system and inducing side effects that impair the patient's quality of life. Because seizure activity represents a subtle functional perturbation of the normal physiologic activity of the nervous system, this goal is difficult to attain. Nevertheless, the identification by Merritt and Putnam in 1938 of phenytoin as the first nonsedating anticonvulsant demonstrated that the goal is achievable (1), and in recent years drug screening in animal models has uncovered a wide variety of chemical compounds with excellent anticonvulsant efficacy and a low incidence of side effects.

To exhibit anticonvulsant activity, a drug must act on one or more target molecules in the brain. These targets may include ion channels, neurotransmitter transporters, and neurotransmitter metabolic enzymes. The ultimate effect of these interactions is to modify the bursting properties of neurons and reduce synchronization in neuronal ensembles. In addition, anticonvulsant drugs inhibit the spread of abnormal firing to distant sites (2). Synchronous epileptiform activity in localized neuronal ensembles is associated with interictal spike activity, but is not sufficient for the expression of behavioral seizure activity. Rather, the expression of partial seizures requires the recruitment and synchronization of a large cortical mass, and the ability to interfere with the spread of epileptiform activity is likely to be an essential feature of anticonvulsant drugs that are useful in the treatment of partial seizures. Similarly, generalized seizures, which by definition are associated with bilateral cortical involvement, are believed to result from thalamocortical synchronization (3). Interference with the rhythm-generating mechanisms that underlie this synchronized activity is necessary to abort these seizures.

The mechanisms of action of most antiepileptic drugs is not known with certainty. All drugs—including antiepileptic drugs—have a diversity of actions on biologic systems,

only some of which are related to the desired therapeutic effect. It often is difficult to select from among the many pharmacologic effects of any drug just those that are relevant to its therapeutic activity in epilepsy. This is largely because the fundamental pathophysiology of epilepsy is incompletely understood, and any alteration of the excitability properties of neurons could potentially be relevant to anticonvulsant effects. Moreover, many anticonvulsant drugs appear to act through multiple complementary mechanisms, and indeed this may be an important way in which anticonvulsant drugs that target critical brain excitability systems are able to protect against seizure activity with relatively low central nervous system toxicity (4). Nevertheless, it is convenient to categorize antiepileptic drug actions according to those that involve (a) modulation of voltage-dependent ion channels, (b) enhancement of synaptic inhibition, and (c) inhibition synaptic excitation. Voltage-dependent ion channels (including sodium, calcium, and potassium channels) shape the subthreshold electrical behavior of the neuron, allow it to fire action potentials, regulate its responsiveness to synaptic signals, contribute to the paroxysmal depolarizing shift (the single-cell correlate of the interictal discharge), and ultimately are integral to the generation of seizure discharges. In addition, voltage-dependent ion channels are critical elements in neurotransmitter release, which is required for synaptic transmission. Consequently, they are key targets for anticonvulsants that inhibit epileptic bursting, synchronization, and seizure spread. Synaptic inhibition and excitation are mediated by neurotransmitter-regulated channels; these channels permit synchronization of neural ensembles and allow propagation of the abnormal discharge to local and distant sites. Anticonvulsants that modify excitatory and inhibitory neurotransmission therefore also can suppress bursting and, when they inhibit synaptic excitation, can have prominent effects on seizure spread.

Which ion channels are relevant to the actions of antiepileptic drugs? Studies in animal models have demonstrated that anticonvulsant effects can be achieved by blockade of sodium or calcium channels, and probably also through facilitation of potassium channels (as may be the case for reti-

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gabine) (5). Anticonvulsant effects are also well known to be produced by drugs that enhance inhibition mediated by γ -aminobutyric acid type A (GABA_A) receptors (and in some cases, also possibly GABA_B receptors), or through effects on glycine systems, the regionally specific transmitter systems (including monoamines such as catecholamines, serotonin, and histamine, and neuropeptides, including opioid peptides and neuropeptide Y), and the inhibitory neuromodulator adenosine (6–8). In addition, blockade of excitatory amino receptors [including those of the *N*-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), metabotropic and possibly also kainate types] also can protect against seizures. In principle, it may be possible to prevent the occurrence of seizures by targeting any one or a combination of these systems. In fact, the development of antiepileptic drugs by screening in animal models that are nonbiased with respect to mechanism has uncovered drugs that act by a number of these mechanisms. In many cases, the relevant mechanisms are not shared by other agents. Indeed, it is safe to say that no two marketed drugs work in exactly the same way. This chapter focuses mainly on those molecular targets relevant to the actions of clinically important antiepileptic drugs. Information on additional drug targets of research interest is available (6).

MOLECULAR TARGETS OF ANTIEPILEPTIC DRUGS

Voltage-Gated Sodium Channels

Overview

Brain voltage-gated sodium channels are the molecular targets of a number of chemically diverse antiepileptic drugs.

All of these drugs act by inhibiting ionic current through the channel, but the precise way in which this results in protection against seizures is incompletely understood (9). Nevertheless, the molecular detail through which prototypical sodium channel blocking anticonvulsants such as phenytoin and lamotrigine interact with sodium channels has been extensively characterized.

Voltage-gated sodium channels are responsible for the rising phase of neuronal action potentials. When neurons are depolarized to action potential threshold by an excitatory synaptic input, the sodium channel protein senses the depolarization and within a few hundred microseconds undergoes a conformational change that converts the channel from the closed (deactivated), nonconducting, resting state to the conducting open state that permits sodium flux. Within a few milliseconds, the channel inactivates, terminating the flow of sodium ions. The channel must then be repolarized before it can be activated again by a subsequent depolarization. Brain sodium channels can rapidly cycle through the resting, open, and inactivated states, allowing brain neurons to fire trains of action potentials at high frequency, as is required for normal brain function and for the expression of epileptic activity.

Sodium Channel Structure

The sodium channel from mammalian brain is a complex of α (260 kd), $\beta 1$ (36 kd), and $\beta 2$ (33 kd) protein subunits (10). The α subunit forms the ion-conducting pore of the channel and also contains the machinery required for voltage-dependent gating. The β subunits are not required for functional activity of the sodium channel, but modulate channel expression and alter the gating properties. α Sub-

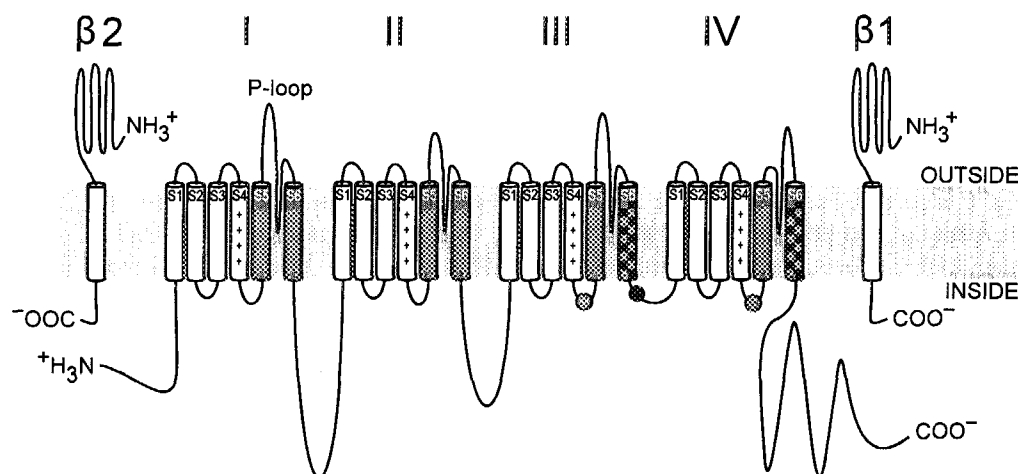


FIGURE 1.1. Primary structures of the subunits of brain type II voltage-gated sodium channels. The main α subunit, consisting of four homologous repeats (I to IV), is shown flanked by the two auxiliary β subunits. Cylinders represent probable α -helical transmembrane segments. Dark gray α -helical segments are believed to form the pore region; P-loops form the outer pore and ion selectivity filter. Plus signs indicate S4 voltage sensors; black circle, inactivation particle in inactivation gate loop ("tethered pore blocker"); and gray circles, sites implicated in forming the receptor for the inactivation gate. III S6 and IV S6 (patterned) are regions of modulatory drug binding, including sodium channel blocking anticonvulsants.

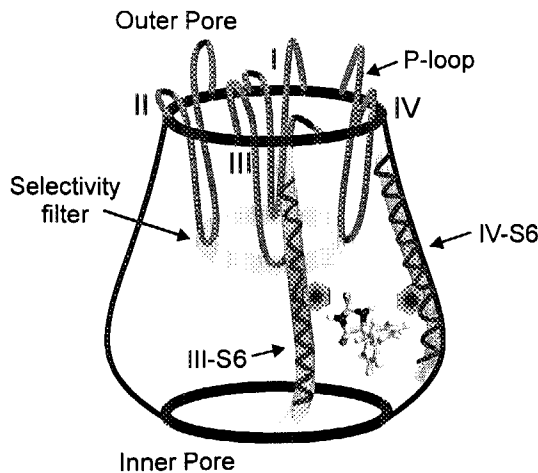


FIGURE 1.2. Schematic illustration of the pore of the voltage-gated sodium channel α -subunit. The S5 and S6 transmembrane α -helical segments from each homologous repeat (I to IV) form the four walls of the pore. The outer pore mouth and ion selectivity filter are formed by reentrant P-loops. The critical α -helical S6 segments in repeat III and IV, which contain the anticonvulsant binding sites (hexagons), are emphasized. A phenytoin molecule is illustrated in association with its binding sites.

units have six α -helical transmembrane segments (S1 to S6) in each of the four homologous domains (I to IV) and a reentrant loop ("P-loop") between transmembrane segments S5 and S6 that dips into the membrane and forms the narrower outer pore and the ion selectivity filter (Figures 1.1 and 1.2). The four subunit-like domains are believed to form a square array in the membrane, with an ion-conducting pore in the center. Each domain contains a number of positively charged amino acid residues in the S4 segment that serve as the voltage sensor that couples membrane depolarization to channel opening and to fast inactivation (11).

Anticonvulsant Modulation of Sodium Channels

Modulation of the gating of brain sodium channels is believed at least in part to account for the anticonvulsant activity of several antiepileptic drugs, including phenytoin, lamotrigine, carbamazepine, oxcarbazepine (12,13), zonisamide (14) and possibly also felbamate (15) and topiramate (16). Antiepileptic agents that act on sodium channels characteristically exhibit protective activity in the maximal electroshock test, a widely used animal model for the screening of anticonvulsant drugs, and they are effective in the treatment of partial and generalized tonic-clonic seizures in humans (9,17,18). These drugs have the unique property that they block high-frequency repetitive spike firing, as is believed to occur during the spread of seizure activity, without affecting ordinary ongoing neural activity (Figure 1.3). This accounts for their ability to protect against seizures without causing a generalized impairment of brain function.

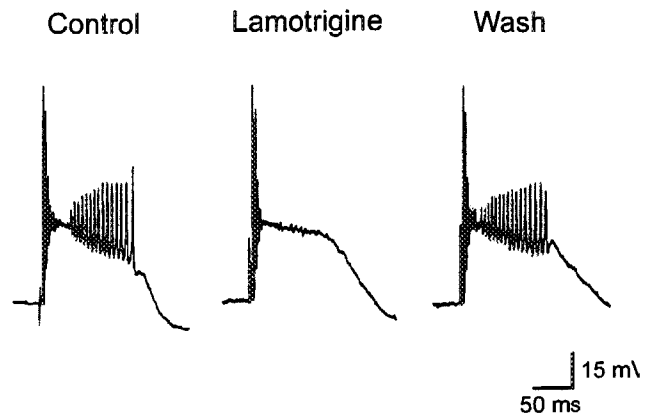


FIGURE 1.3. Lamotrigine (50 $\mu\text{mol/L}$), a sodium channel blocking anticonvulsant, selectively inhibits epileptiform afterdischarges in CA1 hippocampal neurons without affecting initial action potential responses. The intracellular recording was carried out in a rat hippocampal slice under magnesium-free conditions (to enhance NMDA receptor responses) and in the presence of 20 $\mu\text{mol/L}$ bicuculline (to block GABA_A receptors). Synaptic responses were evoked with Schaffer collateral/commissural fiber stimulation. (From Xie X, Lancaster B, Peakman T, et al. Interaction of the antiepileptic drug lamotrigine with recombinant rat brain type IIA Na⁺ channels and with native Na⁺ channels in rat hippocampal neurones. *Pflügers Arch* 1995;430:437–446, with permission.)

An understanding of the basis by which sodium channel anticonvulsants selectively inhibit high-frequency action potential firing has come from voltage-clamp studies, which allow the kinetic properties of ion channel gating and the voltage dependence of drug action to be characterized in detail. These studies have shown that at hyperpolarized membrane potentials, clinically relevant concentrations of drugs such as phenytoin and lamotrigine produce only a weak block of sodium channels (19,20) (Figure 1.4). However, when the membrane is depolarized, there is a marked increase in the degree of tonic inhibition. For example, the inhibitory potency of phenytoin for recombinant type IIA sodium channels (the predominant form in brain neurons) increases more than 100-fold when the membrane is depolarized from -90 to -60 mV (20) (Figure 1.4B). Moreover, the inhibitory potency is strongly "use-dependent," which means that block accumulates with repetitive activation (Figure 1.4C). These properties of block are explained by preferential binding of the drug to inactivated conformations of the channel. For example, the affinity of phenytoin for inactivated sodium channels is of the order of 7 $\mu\text{mol/L}$ —well within the therapeutic concentration in the cerebrospinal fluid of 4 to 8 $\mu\text{mol/L}$ —whereas its affinity for resting sodium channels is >600 $\mu\text{mol/L}$ (21). Similarly, it is estimated that lamotrigine binds to the inactivated state with an affinity of 7 to 12 $\mu\text{mol/L}$, which also is within the estimated therapeutic brain concentration range of 10 to 30 $\mu\text{mol/L}$, whereas the affinity for the resting state is 40- to 200-fold lower (20,22). The effects of these agents at clinically relevant concentrations are mainly on action potential firing; the drug does not directly alter excitatory or

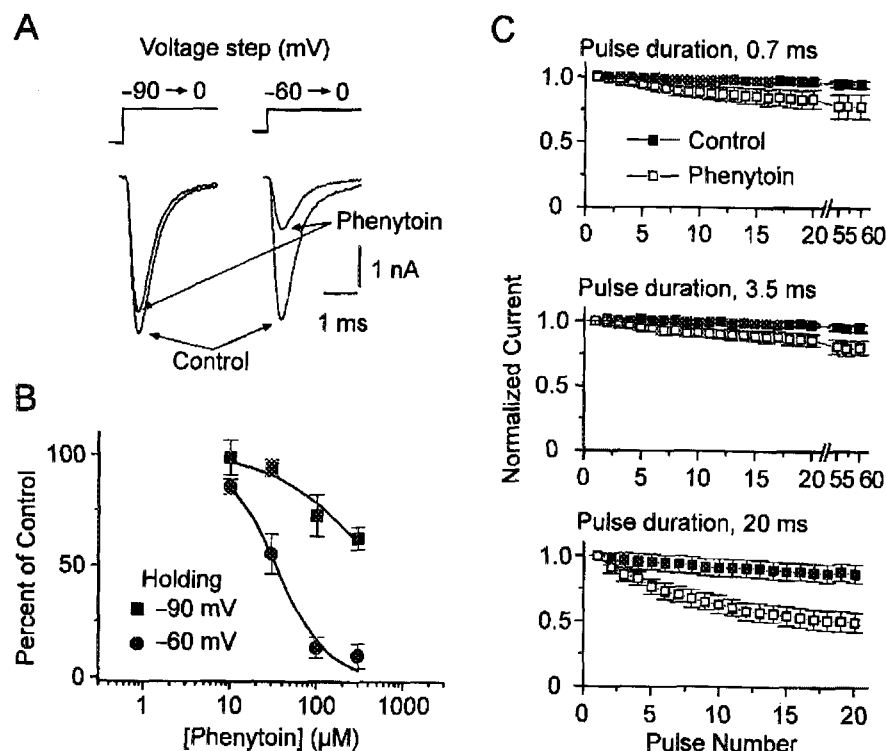


FIGURE 1.4. Voltage-dependent and use-dependent block of human type IIA sodium channels by phenytoin. **A:** Phenytoin ($50 \mu\text{mol/L}$) inhibits sodium current activated from a holding potential of -60 mV more strongly than from a holding potential of -90 mV. **B:** Concentration-response relationship for phenytoin block of peak sodium current activated from the two holding potentials. There is nearly a 15-fold increase in potency when the current is activated from the more depolarized holding potential. **C:** Accumulation of block with repeated sodium channel activation and inactivation. The extent of use-dependent inhibition increases for longer pulse durations, in which the sodium channels are maintained in the inactivated state for longer times. Currents were normalized to the amplitude of the first pulse in the presence of phenytoin, so that tonic block is not represented. Voltage-clamp recordings are from cloned human brain type IIA α subunits stably expressed in Chinese hamster ovary cells. (From Xie X, Dale TJ, John VH, et al. Electrophysiological and pharmacological properties of the human brain type IIA Na^+ channel expressed in a stable mammalian cell line. *Pflügers Arch* 2001;441:425–433, with permission.)

inhibitory synaptic responses. However, the effect on action potentials does ultimately translate into reduced transmitter output at synapses (see later).

In recent years, the mechanism of sodium channel inactivation has been elucidated and this has provided an opportunity to clarify the way in which phenytoin and other sodium channel blocking anticonvulsants appear to promote channel inactivation. The normal predominant (fast) inactivation process results from occlusion of the intracellular mouth of the channel by a short loop of amino acid residues between domains III and IV of the sodium channel α subunit that serves as a "tethered pore blocker" (Figure 1.1). An additional inactivation process referred to as *slow inactivation* begins to come into play with more prolonged depolarizations, such as might occur in association with epileptiform activity. This distinct inactivation mechanism, which is coupled to slow recovery, appears to involve different structural domains of the sodium channel (23), including the outer pore region (24). Phenytoin induces a nonconducting state of the channel that is similar to channel inactivation. This can occur even in sodium channels where fast inactivation has been eliminated by enzymatic removal of the inactivation loop (25). Therefore, phenytoin does not act by stabilizing the normal inactivation state. Rather, phenytoin induces an inactivated state with distinct kinetic properties. Recovery from drug block of the channel occurs much more slowly than does recovery from block by the intrinsic pore blocker (26). This, in part, accounts for phenytoin's unique ability selectively to block high-

frequency firing because, when recovery is slow, block can accumulate during repetitive activation of the channel.

An additional critical feature of phenytoin and lamotrigine block of sodium channels (and one that distinguishes these drugs from local anesthetics) is the slow onset of block. [Rate constants for both phenytoin and lamotrigine are $\sim 10^4 \text{ mol/L}^{-1} \text{ s}^{-1}$ (21,22).] This may result from a strict stereospecific requirement for binding in which only a small fraction of collisions between the drug and the channel acceptor site result in binding (21). However, once binding occurs, it is tight, and unbinding (recovery from block) is slow. Slow binding has two important implications. First, slow binding implies that the time course of sodium currents is not altered in the presence of the drug, and therefore the kinetic properties of action potentials are not perturbed. Second, slow binding means that inhibition of action potentials does not occur with firing induced by synaptic depolarizations of ordinary length. Rather, long depolarizations are required, possibly as long as a few seconds or greater. In focal epilepsies, the cellular events that characterize ictal discharges are sustained depolarizations that evoke intermittent high-frequency bursts of action potentials. Such depolarizations provide the conditions required for drug binding and block. On the other hand, cortical interictal discharges are of shorter duration, and this corresponds with the electroencephalographic concept that such discharges are unaffected by phenytoin. Overall, sodium channel blocking anticonvulsants are expected to have little effect on the physiologic generation of action

potentials in well polarized neurons. The voltage- and use-dependent characteristics of block would come into play only during pathologic sustained depolarizing events associated with high-frequency discharges, thus allowing protection against seizures without interfering with normal function.

Anticonvulsant Binding Domain

Studies with mixtures of phenytoin, carbamazepine, and lamotrigine have revealed that these drugs bind to a common recognition site on sodium channels (27). Although these three compounds are structurally dissimilar, they do contain a common motif of two phenyl groups separated by one to two C-C or C-N single bonds (1.5 to 3 Å) (Figure 1.5). These two phenyl groups probably are critical elements in binding. Phenytoin is active in its neutral, uncharged form. Therefore, binding of phenytoin with its receptor on sodium channels is nonionic and involves hydrophobic or induced-dipole interactions. In rat brain type IIA sodium channels, mutations of specific phenylalanine (1764) and tyrosine (1771) residues in the S6 segment of domain IV—a stretch of amino acids that contributes to the inner lining of the channel pore—dramatically reduces the potency for use-dependent block by phenytoin and local anesthetics. This occurs because of decreased drug affinity for the inactivated channel conformation. Thus, transmembrane segment IVS6 likely contributes to the anticonvulsant and local anesthetic receptor. In addition, mutational analysis has revealed that the pore lining residues leucine 1465 and isoleucine 1469 in IIIS6 also form a portion of the high-affinity binding site for sodium channel blocking anticonvulsants (28). The aromatic rings in the anticonvulsant molecules may interact with the aromatic side chains of the critical residues in the IVS6 segment or with the nonpolar side chains of the IIIS6 residues. It is noteworthy that this region of the sodium channel also is implicated in inactivation gating (29). Gating movements

of the IIIS6 and IVS6 segments may allow access of the anticonvulsants to their receptor site, which becomes available as the channel opens and increases in affinity as it inactivates.

Differences in Kinetics of Block among Sodium Channel Blocking Anticonvulsants

Although carbamazepine, phenytoin, and lamotrigine interact with sodium channels in a similar fashion at the same binding site, there are quantitative differences in the rate and extent of block. Thus, carbamazepine has approximately threefold lower binding affinity for the inactivated state of the channel (apparent dissociation constant, 25 $\mu\text{mol/L}$), and the extent of steady-state block at therapeutic concentrations is estimated to be modestly lower (30). However, the binding rate of carbamazepine is approximately five times faster ($3.8 \times 10^4 \text{ mol/L}^{-1} \text{ s}^{-1}$), so that it might be effective in situations where the ictal depolarizations are shorter. In view of the kinetic differences between drugs, the use of combinations of sodium channel blocking anticonvulsants is perhaps not as illogical as it would seem.

Persistent Sodium Current

In addition to effects on the fast sodium current responsible for action potentials, blockade of persistent (noninactivating) sodium currents also may be an important mechanism of anticonvulsant drug action. Persistent sodium current flows through a portion of the same sodium channels that normally give rise to the fast sodium current but temporarily fail to inactivate for an extended period (31). The current carried by the persistent openings is a minute fraction of the fast current. However, this current may play a key role in regulating excitability near firing threshold because it is largely unopposed by other voltage-activated currents in this range of membrane potentials. Moreover, there is evidence that the persistent sodium current con-

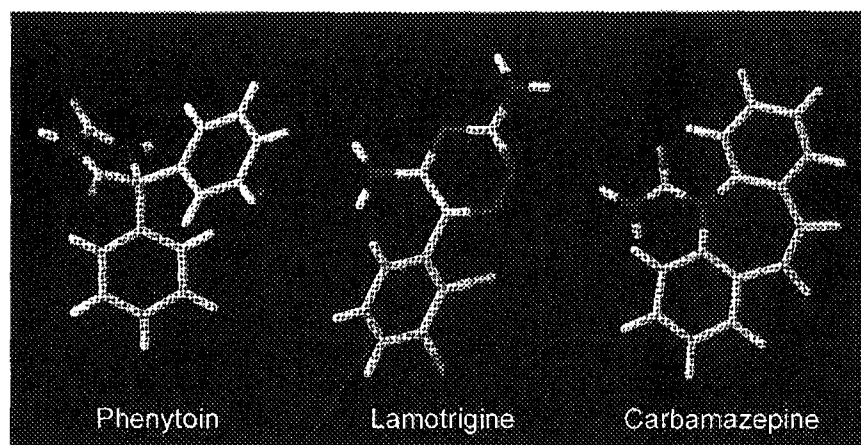


FIGURE 1.5. Comparison of the three-dimensional structures of sodium channel blocking anticonvulsants. The anticonvulsants are structurally dissimilar: phenytoin is a hydantoin (a cyclic ureide); lamotrigine is a dichlorophenyl-triazine; and carbamazepine is a dibenzazepine that is structurally related to the tricyclic antidepressants. The structures are shown to indicate the similar but not superimposable orientations of the phenyl rings (1,2,4-triazine ring in lamotrigine), suggesting the comparable ways in which the drugs may bind to sodium channels.

tributes to the initiation and maintenance of epileptiform activity (32). Several authors have reported that phenytoin (32–34) and topiramate (16) inhibit persistent sodium current at concentrations lower than those that block fast sodium current. The selective reduction of late sodium channel openings may contribute to the ability of these drugs to protect against seizures with minimal interference in normal function.

GABA Systems

Potentiation of inhibitory neurotransmission is a key mechanism of antiepileptic drug action. Although a variety of neurotransmitters, including biogenic amines (e.g., norepinephrine, serotonin) and neuropeptides (e.g., opioid peptides, neuropeptide Y), can mediate inhibition of neuronal excitability through presynaptic or postsynaptic mechanisms, GABA is the major inhibitory neurotransmitter in the forebrain. Neurons that use GABA as their neurotransmitter—mainly interneurons—represent only a small fraction of central nervous system neurons, and in cortical regions critical to epileptogenesis, excitatory synapses may be severalfold more common anatomically than inhibitory ones. However, these inhibitory connections are critically important in restraining the natural tendency of recurrently connected excitatory neurons to transition through positive feedback into synchronized epileptiform discharges.

All clinically relevant antiepileptic drugs that enhance inhibition do so through an action at GABA synapses. GABA acts through fast Cl^- -permeable ionotropic GABA_A receptors and also through slower metabotropic G-protein-coupled GABA_B receptors. Reduction in the efficacy of synaptic inhibition mediated by GABA_A receptors—for example, with drugs that block GABA_A receptors, like bicuculline and pentylenetetrazol—can lead to seizures. Moreover, rare naturally occurring mutations in the γ subunit of GABA_A receptors have been associated with increased seizure susceptibility in humans (35,36). Conversely, pharmacologic enhancement of GABA_A receptor-mediated synaptic inhibition is an effective anticonvulsant approach. This can occur through a direct action on GABA_A receptors, or indirectly by blockade of the enzymes and transporters responsible for terminating the synaptic actions of GABA. Drugs that act through these mechanisms typically have a broad spectrum of anticonvulsant activity in human seizure disorders, although—with the exception of benzodiazepine receptor agonists—they usually are ineffective in absence seizures.

GABA_A Receptors

Overview

GABA_A receptors are members of the ligand-gated ion channel superfamily, which includes nicotinic acetyl-

choline, 5-HT₃, and strychnine-sensitive glycine receptors (37,38). Members of this superfamily are heterooligomeric pentamers (Figure 1.6). The protein subunits that constitute the pentamer in GABA_A receptors have a large extracellular amino terminus, four hydrophobic transmembrane domains (M1–4), and a large intracellular domain between M3 and M4. GABA_A receptor subunit proteins are classified into subfamilies termed α , β , γ , δ , ϵ and θ (Figure 1.7). There are six α subunits, four β subunits (with two splice variants), three γ subunits (with two splice variants), and one each of δ , ϵ , and θ . In addition, there are two ρ subunits (GABA_C receptors), mainly expressed in the retina, that do not coassemble with other subfamily members and have unique pharmacologic properties. Although more than 2,000 combinations of subunits are theoretically possible, most GABA_A receptors are believed to be composed of α , β , and γ subunits with stoichiometry of 2:2:1. The δ , ϵ , and θ subunits can replace the γ subunit in some receptor subtypes.

The pharmacology of GABA_A receptors is the best developed and most complex of any of the ligand-gated ion channels. A large number of structurally specific recognition sites for drugs and chemicals have been identified on the receptor-channel complex. However, from the point of view of clinically relevant anticonvulsant drug actions, modulation by benzodiazepine-like agents and barbiturates is of most significance, although felbamate (39) and topiramate (40,41) may also act, in part, through effects on GABA_A receptors. Moreover, there is emerging evidence that neurosteroids—endogenous metabolites of progesterone that act as barbiturate-like modulators of GABA_A receptors—may contribute to hormonal influences on seizure susceptibility (42).

Benzodiazepines

Benzodiazepine receptor agonists, such as diazepam and lorazepam, do not directly activate GABA_A receptors. Rather, they act as positive allosteric modulators to enhance the action of GABA by increasing the frequency of channel openings induced by GABA (43) [and possibly by increasing the single-channel conductance of low-conductance channels (44)]. The sensitivity of GABA_A receptors to benzodiazepines is highly subunit dependent (45). The $\gamma 2$ subunit is required for full benzodiazepine-positive allosteric modulation. Benzodiazepine activity is reduced when $\gamma 2$ is replaced by $\gamma 1$ or $\gamma 3$, and eliminated if γ is absent or is replaced by δ or ϵ . The critical nature of the $\gamma 2$ subunit for benzodiazepine modulation is demonstrated by the nearly complete lack of benzodiazepine activity in $\gamma 2$ subunit-deficient mice (46).

Benzodiazepine modulation also depends on the α subunit. GABA_A receptors containing the $\alpha 4$ and $\alpha 6$ subunits do not respond to classic benzodiazepines such as diazepam. These subunits have an arginine instead of a histidine at a

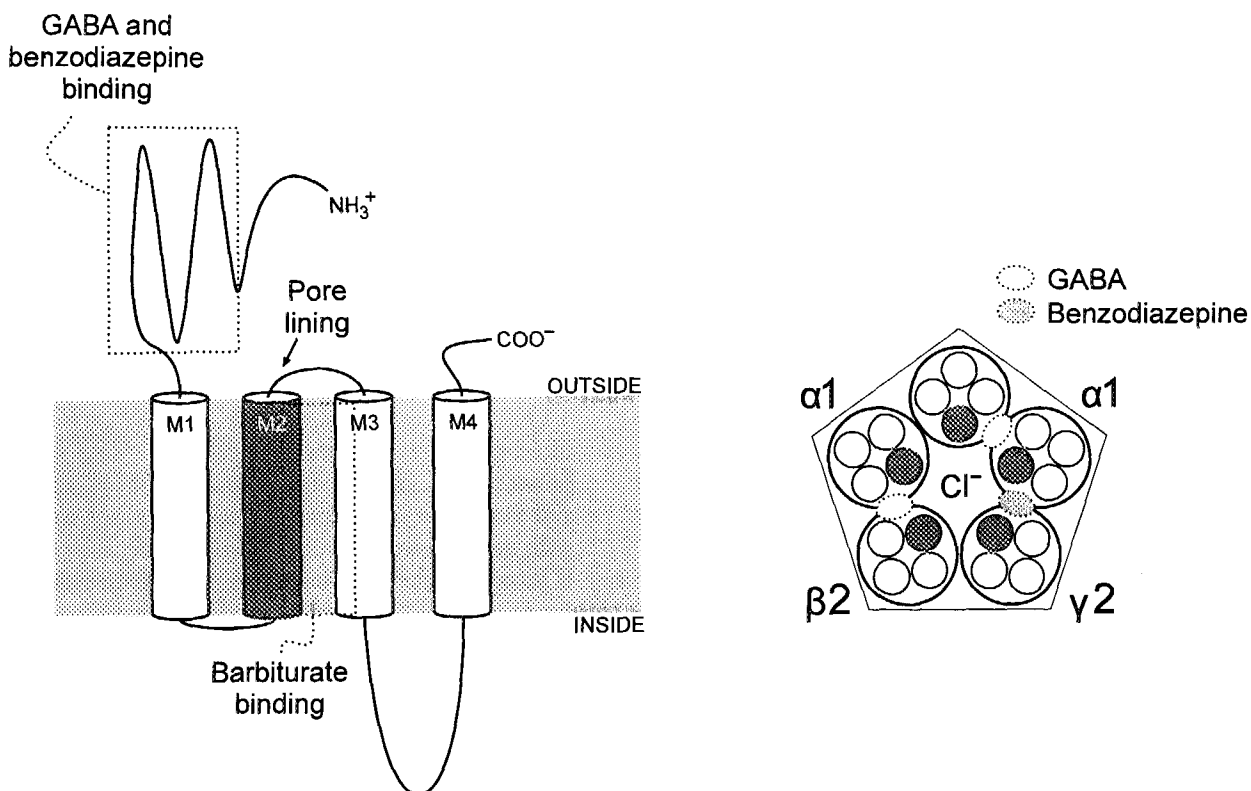


FIGURE 1.6. Left: Primary structure and membrane topology of GABA_A receptor subunits. Residues identified as critical for agonist (GABA) and benzodiazepine binding are in the extracellular amino terminus. The specific residues contributing to binding of the two types of ligands are distinct. Barbiturate binding occurs to the membrane-spanning M2 and M3 segments. **Right:** Schematic illustration of the pentameric structure of GABA_A receptors. Small circles represent membrane-spanning α -helical segments corresponding to those shown in diagram at left. M2 segments form the Cl⁻ channel pore. Agonist and benzodiazepine binding domains are at homologous positions at subunit interfaces. There are two agonist binding sites and a single benzodiazepine binding site in each GABA_A receptor complex. The $\alpha 1\beta 2\gamma 2$ subunit configuration represents a common GABA_A receptor type.

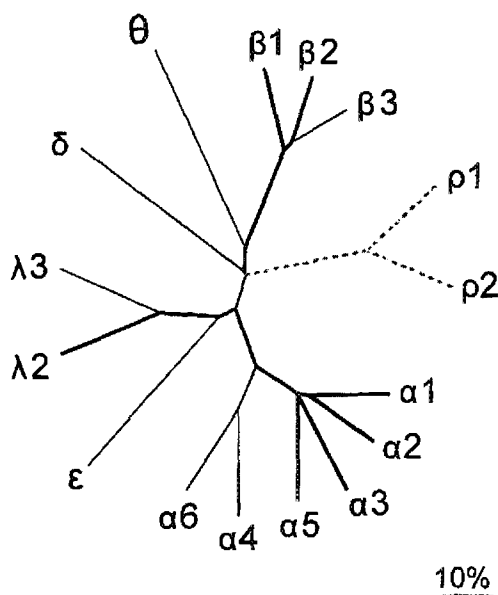


FIGURE 1.7. Radial tree illustrating homology between the 16 cloned human GABA_A receptor subunits. The most abundant subunits in brain are identified by thick line segments. Homomeric receptors composed of ρ subunits, which are expressed primarily in retina, have distinct pharmacologic properties similar to GABA_C receptors as defined by Johnston (167); the extent to which these subunits form heteromeric assemblies with conventional GABA_A receptor subunits is uncertain (168). Homology analysis was produced with ClustalW using NCBI RefSeq records. Scale bar indicates 10% identity.

conserved position, which in the $\alpha 1$ and $\alpha 2$ subunits is position 101. Replacement of this histidine with arginine in the $\alpha 1$ subunit markedly reduces the anticonvulsant (and also sedative and amnestic) activity of diazepam, but does not alter the anxiolytic properties, indicating that the anticonvulsant activity of benzodiazepines in part involves receptors containing the $\alpha 1$ subunit, whereas the anxiolytic actions seem to be mediated by receptors that contain $\alpha 2$, $\alpha 3$, or $\alpha 5$ subunits (47,48). Mutational analysis has further indicated that the benzodiazepine binding pocket of the GABA_A receptor lies at the interface between the α and γ subunits, with residues from each subunit contributing to the benzodiazepine recognition site (49) (Figure 1.6). This site is in a homologous position to the two agonist (GABA) binding sites at the interface between other subunits.

In clinical practice, benzodiazepines have an important role in the acute treatment of status epilepticus. However, two significant limitations largely preclude their use in chronic therapy. The first limitation is the undesirable side effects, including sedation and muscle relaxation, that occur at doses comparable with those that protect against seizures. The second, and probably more important limitation (because side effects tend to diminish in time) relates to the tolerance that develops with chronic use. In animal models, benzodiazepine receptor partial agonists can exhibit anticonvulsant activity without sedative actions (6). These agents also may have reduced tolerance liability. However, in human trials none of these agents has been sufficiently free of side effects and tolerance liability to be clinically useful (50). The development of benzodiazepine receptor ligands that selectively target GABA_A receptor subtypes in a way that confers anticonvulsant activity and avoids these limitations is a goal that has yet to be realized. However, compounds such as AWD 131-138 [1-(4-chlorophenyl)-4-morpholino-imidazolin-2-one], a weak partial benzodiazepine receptor agonist currently in development that seems to lack tolerance liability, may hold promise (51).

Benzodiazepines and Absence Seizures

Hypersynchronous activity in thalamocortical circuits is believed to underlie the 3-Hz spike-and-wave activity characteristic of generalized absence seizures (3,52). The well recognized antiabsence activity of benzodiazepines, such as clonazepam, likely resides in their ability to "desynchronize" these oscillations. Thalamocortical relay neurons and corticothalamic neurons form a mutually excitatory loop whose oscillatory behavior is regulated by inhibitory GABAergic connections to relay neurons from the reticular thalamic nucleus (RTN). In addition to inhibiting relay neurons, RTN neurons send recurrent collaterals to neighboring inhibitory neurons in the RTN. This intranuclear inhibition normally diminishes synchronous firing in the RTN and reduces the inhibitory output below that required for absence seizure activity (53). GABA_A receptors on RTN neurons appear to be highly sensitive to benzodiazepines,

possibly more so than GABA_A receptors on relay neurons. Thus, by selectively enhancing the strength of recurrent inhibition in the RTN, benzodiazepines may promote endogenous antiabsence mechanisms.

Phenobarbital, Felbamate, and Topiramate

Barbiturates, including phenobarbital, also act as positive allosteric modulators of GABA_A receptors, but this occurs in a way distinct from the action of benzodiazepines (Figure 1.8A). GABA-activated chloride channels open in "bursts," interrupted by frequent brief closures. The openings in bursts can be classified into groups with brief, intermediate, and long average durations; longer openings are associated with more prolonged bursts. As the concentration of GABA is increased, the channels open more frequently and enter the longer-lived open states proportionately more often. At clinically relevant concentrations, phenobarbital does not increase the frequency of GABA-induced channel opening, but rather shifts the relative proportion of openings to favor the longest-lived open state associated with prolonged bursting, thus increasing the overall probability that the channel is open (37). In addition, barbiturates have actions on other ion channel systems, including calcium (see later) and sodium channels (54), that likely contribute to their therapeutic activity and may also be a factor in side effects. Apart from their modulatory actions on GABA_A receptors, barbiturates (unlike benzodiazepines) directly activate GABA_A receptors in the absence of GABA (55) (Figure 1.8B). This direct action, which occurs at higher concentrations than the modulatory action, also is likely to contribute to the sedative side effects of phenobarbital. The single-channel conductance of GABA_A receptor channels is similar when the channels are activated by GABA, barbiturates, and the two together, indicating that the structure of the pore is similar when the gate is opened by GABA or barbiturates. However, the macroscopic and single-channel currents directly activated by barbiturates have kinetic properties distinct from barbiturate-potentiated GABA-activated currents (55). Moreover, mutation of a single amino acid in the M2 segment of the $\beta 1$ subunit eliminates barbiturate potentiation but does not interfere with direct activation (56). Thus, the modulatory and direct actions involve distinct mechanisms.

Felbamate also acts as a weak barbiturate-like positive allosteric modulator of GABA_A receptors (39) (Figure 1.8C). In contrast to barbiturates, felbamate does not activate GABA_A receptor chloride current responses in the absence of GABA, thus perhaps contributing to its lack of sedative side effects. Moreover, both phenobarbital (55,57) and felbamate (39) can block GABA_A receptors at high concentrations. This would be expected to limit the extent of positive modulation (resulting in a partial agonist-like effect), and also could contribute to the reduced tendency of these drugs to produce sedation at anticonvulsant doses (compared with agonists that have greater efficacy, such as

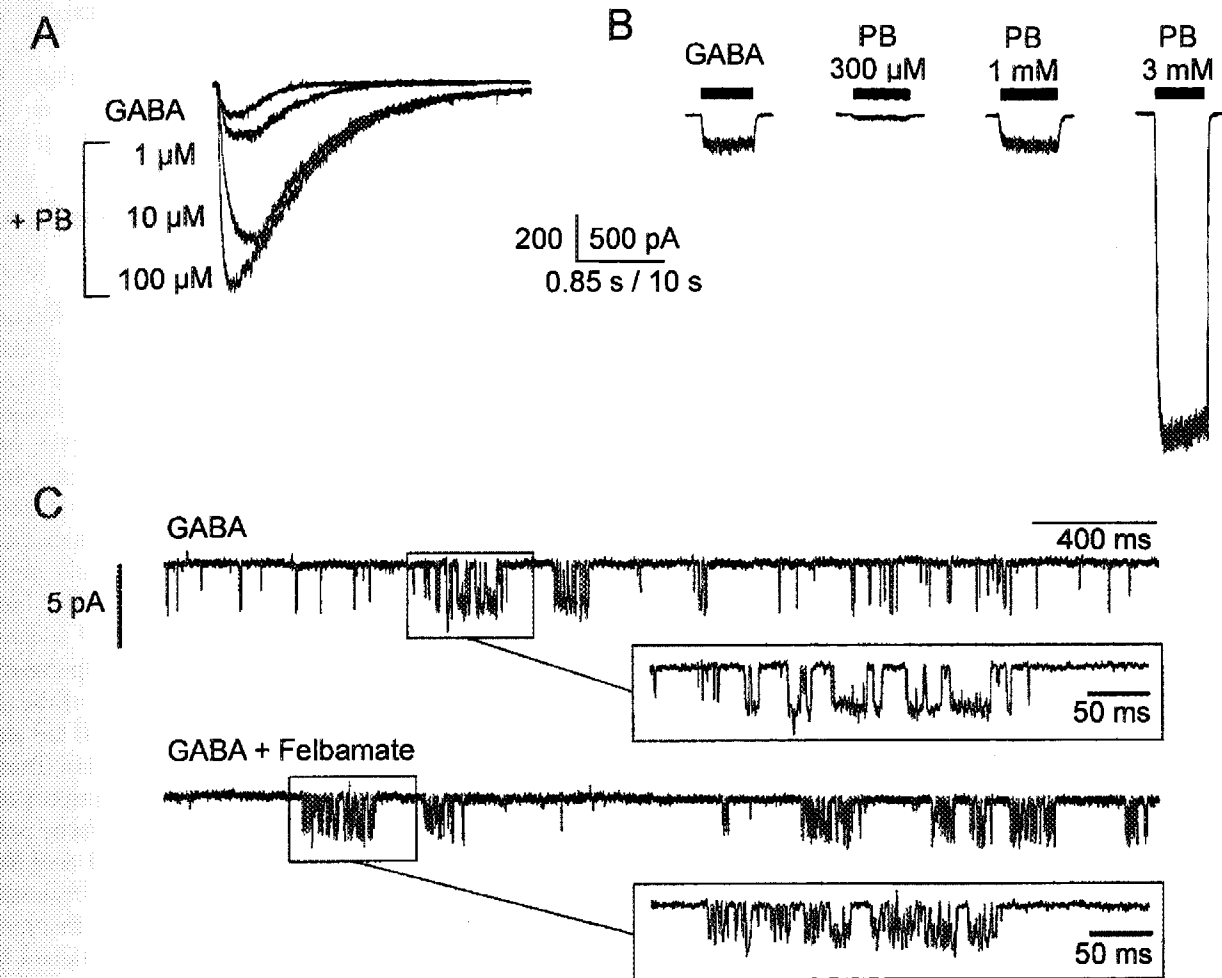


FIGURE 1.8. Modulatory and direct actions of phenobarbital (PB) and felbamate on GABA $_A$ receptors in hippocampal neurons. **A:** PB enhances GABA-activated Cl^- current responses. Whole-cell voltage-clamp recording showing family of currents activated by 50 ms-duration pulses of 1 $\mu\text{mol/L}$ GABA. Membrane potential, -50 mV. (From ffrench-Mullen JM, Barker JL, Rogawski MA. Calcium current block by (-)-pentobarbital, phenobarbital, and CHEB but not (+)-pentobarbital in acutely isolated hippocampal CA1 neurons: comparison with effects on GABA-activated Cl^- current. *J Neurosci* 1993;13:3211–3221, with permission.) **B:** Whole-cell voltage clamp recording showing direct activation of GABA receptors by high concentrations of PB in the absence of GABA. For comparison, the response to 1 $\mu\text{mol/L}$ GABA is shown. Membrane potential, -60 mV. [From Rho JM, Donevan SD, Rogawski MA. Direct activation of GABA $_A$ receptors by barbiturates in cultured rat hippocampal neurons. *J Physiol (Lond)* 1996;497:509–522, with permission.] **C:** A single GABA $_A$ receptor channel in an outside-out membrane patch activated by 2 $\mu\text{mol/L}$ GABA exhibits brief openings and some flickery bursts (open is downward). Burst openings are more frequent and prolonged in the presence of 3 mmol/L felbamate. Felbamate did not activate GABA $_A$ receptor channels in the absence of GABA. Membrane potential, -80 mV. (From Rho JM, Donevan SD, Rogawski MA. Barbiturate-like actions of the propanediol dicarbamates felbamate and meprobamate. *J Pharmacol Exp Ther* 1997;280:1383–1391, with permission.)

pentobarbital or meprobamate). Topiramate also may act, in part, as an allosteric modulator of GABA receptors, but its complex subunit-specific actions have not been fully characterized (40,41).

GABA Transaminase

The concentration of GABA in the brain is controlled by two pyridoxal-5'-phosphate-dependent enzymes, gluta-

mate decarboxylase (GAD) and GABA transaminase (GABA-T). GAD, a cytosolic enzyme localized to GABAergic neurons, catalyzes the synthesis of GABA, whereas GABA-T catabolizes the conversion of GABA to succinic semialdehyde. The transamination can take place only if α -ketoglutarate is the acceptor of the amine group. Thus, the transamination is coupled to the synthesis of the GABA precursor glutamate: For every molecule of GABA destroyed, one molecule of precursor is formed (Figure 1.9).

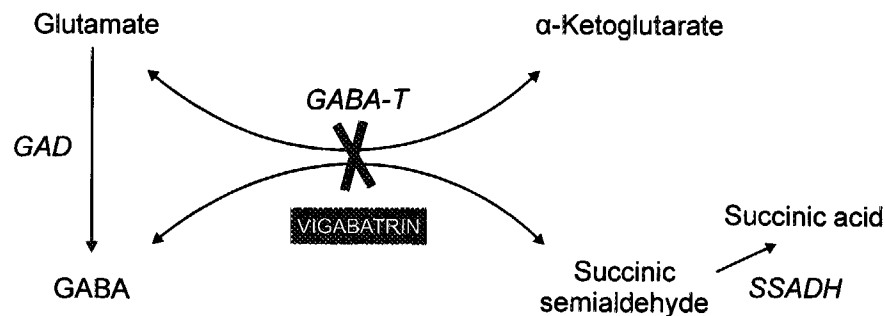


FIGURE 1.9. Metabolism of GABA through the GABA-shunt of the tricarboxylic acid (Krebs) cycle. Degradation of GABA by GABA transaminase (GABA-T; 4-aminobutyrate:2-oxoglutarate aminotransferase) is coupled to the synthesis of the precursor glutamate. Vigabatrin inactivates GABA-T, leading to elevated brain GABA levels. GAD, glutamic acid decarboxylase; SSADH, succinic semialdehyde dehydrogenase.

GABA-T, the product of a single gene, is a mitochondrial enzyme that is expressed widely in brain neurons and glia, and also in peripheral organs (58).

The anticonvulsant vigabatrin (γ -vinyl GABA) is a GABA analog that acts as an irreversible suicide inhibitor of GABA-T (59). The drug initially binds reversibly to the pyridoxal-5'-phosphate cofactor and then irreversibly to the enzyme. Administration of vigabatrin leads to large elevations in brain GABA levels (60–62). The anticonvulsant properties of vigabatrin have been attributed to enhanced GABA-mediated inhibition. Although vigabatrin can cause increased GABA release under some circumstances (63), there is little evidence that it causes a generalized increase in inhibition, and it is not as strongly sedating as GABA receptor modulators such as clonazepam. It has been shown that vigabatrin prevents the fading of inhibitory mechanisms during repetitive stimulation of interneurons and thus preferentially enhances inhibition in a frequency-dependent fashion (64). This effect may occur through a reduction in the sensitivity of the GABA_B receptor mechanisms that normally cause activity-dependent depression of inhibition. Such fading of inhibition is believed to be an important factor that permits focal epileptiform activity to develop into a full-blown seizure (2), and interference with this process could account for the selective suppression of seizures by vigabatrin. An alternative explanation for the anticonvulsant activity of vigabatrin is that it induces a reversal of the GABA transporter, resulting in spontaneous GABA efflux that causes a generalized increase in inhibitory tone (65).

GABA Transporters

The synaptic action of GABA is terminated by its rapid reuptake into presynaptic terminals and surrounding glia by

high-affinity plasma membrane GABA transporters (66). The GABA transporters, which are members of the superfamily of 12-membrane-segment transporters, are electrogenic and require Na^+ and Cl^- for their activity. Transport of one molecule of GABA involves the cotransport of two Na^+ ions and one Cl^- ion. Four GABA transporter proteins have been identified: GAT-1, GAT-2, GAT-3, and BGT-1 (betaine/GABA transporter 1), with distinct regional and cellular localizations in brain (67). GAT-1, the first member of the family to be identified, is the most abundant of the transporters. It is distributed ubiquitously in the nervous system, where it is localized primarily to GABA neurons but also is found in astrocytic processes (68).

In the mid-1970s, the muscimol analog (*R*)-nipecotic acid was identified as a specific GABA uptake inhibitor (69). Nipecotic acid was recognized as having anticonvulsant activity, but only when administered directly into the brain; as a hydrophilic amino acid, it does not effectively penetrate the blood–brain barrier (70). To overcome this problem, a variety of lipophilic analogs were synthesized, including tiagabine, which currently is marketed for the treatment of epilepsy. Tiagabine is a potent and selective competitive inhibitor of GAT-1. The drug binds with high affinity to the transporter, preventing GABA uptake without itself being transported. By slowing the reuptake of synaptically released GABA, tiagabine prolongs inhibitory postsynaptic potentials (71–73) and has a broad spectrum of activity in animal models of epilepsy (74). The prolongation of inhibitory GABA-mediated responses by tiagabine may be enhanced with repetitive activation, as is expected to occur during the synchronous discharge of interneurons associated with epileptic activity. This may minimize the behavioral depression that would accompany indiscriminate enhancement of GABA inhibition. GABA

uptake blockers have been identified that affect a broader range of GABA transporters or that specifically inhibit GABA transporters other than GAT-1 (75,76). These compounds also exhibit anticonvulsant activity, but may have different spectrums of activity in animal seizure models.

GABA_B Receptors

Overview

Fast synaptic GABA_A receptor-mediated inhibition often is followed by a slower, late inhibitory response to GABA mediated by metabotropic seven transmembrane domain G-protein-coupled GABA_B receptors (77,78). Presynaptic GABA_B receptors on axon terminals inhibit P/Q and N-type calcium channels, resulting in decreased evoked neurotransmitter release. In contrast, postsynaptic GABA_B receptor activation causes opening of inwardly rectifying potassium channels, leading to membrane hyperpolarization and inhibition of neuronal excitability. In addition, postsynaptic GABA_B receptors negatively coupled to voltage-dependent calcium channels may serve to inhibit calcium channel-dependent burst discharges. Two major human GABA_B receptor genes have been identified: GABA_{B1} (gb1) and GABA_{B2} (gb2), with the GABA_{B1} gene encoding two structurally distinct N-terminal variants, GABA_{B1a} and GABA_{B1b}. Functional GABA_B receptors result from heterodimerization of one of the GABA_{B1} variants with GABA_{B2}.

Activation of GABA_B receptors with the nonselective agonist baclofen produces anticonvulsant effects in some systems (79,80) and proepileptic effects in others (81). In addition, there are case reports of baclofen-induced convulsive seizures in humans (82), although baclofen in general has little overall effect on seizure frequency (83). The proepileptic effects are believed to occur because of presynaptic, GABA_B receptor-mediated suppression of GABA release from inhibitory interneurons, leading to disinhibition (84). The reduction of inhibition is greater than the suppressive effect on synaptic excitation, so that there is a net increase in excitability.

Gabapentin

Evidence suggests that the anticonvulsant gabapentin may act in part through a specific interaction with postsynaptic GABA_B receptors. Gabapentin, the lipophilic 3-cyclohexyl analog of GABA, was originally synthesized in an attempt to develop a brain-penetrant GABA agonist. In fact, gabapentin is actively transported by the system L transporter, allowing it to enter the brain readily (85). Although gabapentin did not have the expected activity at GABA_A receptors, it has been reported that therapeutically relevant concentrations (15 μ mol/L) selectively activate GABA_{B(1a,2)} heterodimers coupled to inwardly rectifying potassium

channels (86). In at least some central nervous system regions (including the hippocampus), these GABA_B receptors may be predominantly expressed postsynaptically (78). Thus, the selective action of gabapentin on GABA_{B(1a,2)} receptors to induce potassium channel activation may endow the drug with anticonvulsant activity and eliminate the proconvulsant effects associated with nonselective GABA_B receptor activation. In addition, there may be actions on N- and or P/Q-type voltage-dependent calcium channels coupled to GABA_{B(1a,2)} receptors (87) that could suppress epileptiform burst discharges and, in brain regions with presynaptic GABA_{B(1a,2)} receptors, reduce glutamate release from nerve terminals (88). However, although gabapentin does in fact appear to have baclofen-like actions *in vivo*, these effects are not reversed by GABA_B antagonists, indicating that other mechanisms may be more important (89,90). Alternatives include direct effects on calcium channels (see later) or adenosine triphosphate-sensitive potassium channels (91), or effects on GABA metabolism (92).

GABA_B Receptors in Absence Epilepsy

GABA_B receptors also appear to play a role in generalized absence seizures, with GABA_B agonists such as baclofen increasing the frequency and duration of spike-and-wave discharges in several models of absence seizures (93,94), and GABA_B antagonists exerting strong antiabsence effects (95,96). These pharmacologic observations support the concept that GABA_B receptors are critical to the generation of the 3-Hz rhythmic oscillations in the thalamocortical network that underlie absence seizures (97). GABA_B receptor antagonists represent a potential therapeutic approach in generalized absence. However, because these agents may provoke convulsive seizures, caution is warranted (96).

Calcium Channels

Overview

Voltage-gated calcium channels, like sodium channels, are multisubunit protein complexes that permit ion flux when gated open by membrane depolarization (98). However, there is a larger number of functional calcium channel types with a correspondingly greater diversity of functional roles in neurons. Calcium channels are broadly grouped into high-voltage- and low-voltage-activated families. High-voltage-activated channels—which are further subgrouped as L, R, P/Q, and N types—are largely responsible for the regulation of calcium entry and neurotransmitter release from presynaptic nerve terminals. They represent potential anticonvulsant targets because blockade of these channels inhibits neurotransmitter release (99). However, as yet, there are no practical anticonvulsants that specifically target these channels, with the possible exception of gabapentin, which binds with high affinity to certain calcium channel subunits.

High-voltage activated calcium channels consist minimally of an $\alpha 1$ protein (encoded by one of seven genes) that forms the channel pore and voltage sensor (100). A variety of auxiliary subunits associate with the $\alpha 1$ subunits, including β (four genes), $\alpha 2\delta$ (three), and γ (six) subunits. The members of the $\alpha 2\delta$ family include $\alpha 2\delta$ -1, which is ubiquitously expressed; $\alpha 2\delta$ -2, which is expressed in brain and heart; and $\alpha 2\delta$ -3, which is brain specific. $\alpha 2\delta$ -1 consists of two proteins that are coded by a single gene, the product of which is posttranslationally cleaved from a single polypeptide precursor. The auxiliary subunits serve to enhance incorporation of functional channels in the cell membrane and also allosterically alter their kinetic properties and affect drug modulation.

Gabapentin

There is emerging evidence that among its complex actions gabapentin may act, at least in part, through a selective interaction with voltage-dependent calcium channels. Gabapentin binds with high affinity and specificity to the $\alpha 2\delta$ subunit (101) and can block high-voltage-activated calcium currents in brain (102) and sensory (103) neurons. In addition, the drug appears to suppress glutamate release, possibly through an effect on P/Q-type voltage-dependent calcium channels (88). However, the drug does not affect all calcium currents (104), even those in cells expressing $\alpha 2\delta$ subunits (105). [$\alpha 2\delta$ Subunits are ubiquitously coassembled in many voltage-gated calcium channel types and do not determine subgroup type (100); therefore, factors other than $\alpha 2\delta$ binding must determine P/Q specificity.] Gabapentin has been found to bind only to the $\alpha 2\delta$ -1 and $\alpha 2\delta$ -2 subunits, and not to $\alpha 2\delta$ -3 (106). Moreover, it has nearly threefold higher affinity for $\alpha 2\delta$ -1, thus providing a possible basis for its cell type specificity. There also is evidence that the $\beta 2$ subunit interactions may be important for gabapentin actions (105). Nevertheless, the extent to which effects on calcium channels contribute to the anticonvulsant activity of gabapentin remains to be determined.

Lamotrigine

In addition to its actions on voltage-activated sodium channels, lamotrigine also can inhibit high-voltage-activated (N-, P/Q-type) calcium channels (107,108); it does not affect low-voltage-activated T-type calcium channels (109). The importance of calcium channel blockade for lamotrigine's anticonvulsant properties is not well delineated, but this action, along with the sodium channel blockade, could contribute to its effects on neurotransmitter release (see later).

Phenobarbital

Barbiturates are well known to block voltage-activated calcium channels (110). For phenobarbital, block of calcium channels may contribute to the anticonvulsant activity,

but effects on GABA_A receptors are likely of greater importance (111).

T-Type Calcium Channels

Low-voltage-activated (T-type) calcium channels are believed to play a role in the regulation of neuronal firing by participating in bursting and intrinsic oscillations (112). In thalamus, these channels are critical to the abnormal oscillatory behavior that underlies generalized absence seizures. The T-type calcium channel family (CavT) consists of the $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$ subunits, which are ~30% homologous to high-voltage-activated subunits in their putative membrane-spanning regions (113). Thalamic relay neurons express high levels of $\alpha 1G$ subunits, whereas thalamic reticular neurons (GABAergic interneurons that modulate and synchronize thalamic output) express high levels of $\alpha 1I$ and moderate levels of $\alpha 1H$ subunits (114). It has been proposed that the antiabsence seizure activity of ethosuximide is due to blockade of T-type calcium channels in thalamic neurons at clinically relevant concentrations of the drug (115–118). However, this concept has been challenged by Leresche et al. (119), who have proposed that ethosuximide suppresses thalamic bursting mainly by inhibiting a persistent component of the sodium current (I_{NaP}) in thalamic neurons without affecting the fast (inactivating) sodium current that constitutes the bulk (97.5%) of the total sodium current. By enhancing the efficacy of bursting and reducing the delay between depolarization and the onset of burst firing, I_{NaP} in thalamic neurons works in concert with the T-type calcium current and plays a pivotal role in burst generation (120). Therefore, it is a plausible target for the action of ethosuximide. More recent studies have suggested that the action of ethosuximide on T-type calcium currents may be more complex than previously thought, with effects on the persistent component of particular importance (118,121,122). Other antiabsence agents also inhibit T-type calcium channels, including the active metabolites of methosuximide (α -methyl- α -phenylsuccinimide) and tridione (dimethadione). Zonisimide may also block T-type calcium channels (123,124).

Glutamate Receptors

NMDA and AMPA Receptors

Ionotropic glutamate receptors of the NMDA and AMPA types are potentially important anticonvulsant targets (6,125–128). These glutamate-gated cation channels mediate the bulk of fast excitatory neurotransmission in the central nervous system (129). Blockade of either of these classes of glutamate receptors is well recognized to protect against seizures in *in vitro* and *in vivo* models. However, no selective glutamate receptor antagonist has proven to be of practical use in epilepsy therapy, largely because agents examined to date have exhibited unacceptable side effects (or were admin-

istered at subtherapeutic doses). Nevertheless, weak blockade of excitatory amino acid receptors may be one of a complex of multiple actions through which some clinically useful anticonvulsant drugs protect against seizures, and low-affinity NMDA receptor antagonists have clinical potential (129). In addition, selective AMPA receptor antagonists, such as 2,3-benzodiazepines like talampanel (GYKI 53405; LY 300164), are highly effective anticonvulsants in animal models (131) and have shown promise in early clinical trials.

The best evidence among marketed anticonvulsant drugs for such glutamate receptor blocking actions comes from work with felbamate, which has been shown to inhibit NMDA receptors at clinically relevant concentrations (132). Single-channel recording studies are consistent with a functional channel blocking action of the drug or allosteric effects on channel gating. NMDA receptors in mammalian neurons are heterooligomers formed by coassembly of an obligatory NR1 subunit and at least one type of NR2 subunit (128). Felbamate is modestly more potent as an antagonist of NMDA receptors containing NR2B subunits (133,134). Unlike the NR2A subunit, which is distributed ubiquitously in the central nervous system, expression of the NR2B subunit in the adult is restricted largely to the forebrain (135). It has been proposed that NR2B selectivity could in part contribute to felbamate's low neurobehavioral toxicity in relation to other NMDA receptor antagonists because the drug may target NMDA receptor-mediated synaptic transmission in forebrain areas critical to seizure generation and avoid perturbing nonforebrain structures that could mediate side effects. In addition, NR2B subunits are expressed in high abundance in the immature brain, and this could also account for felbamate's clinical utility in childhood seizure disorders, such as the Lennox-Gastaut syndrome.

Kainate Receptors

The kainate receptors are ionotropic glutamate receptors that share ~40% homology with AMPA receptors and ~20% homology with NMDA receptors (136). Like AMPA and NMDA receptors, kainate receptors are responsible for a portion of glutamate-mediated excitation at some synapses, including those in limbic regions relevant to epilepsy (137). In addition to contributing to postsynaptic excitation, kainate receptors on presynaptic axon terminals serve to modulate glutamate release from excitatory afferents, and they also may have the unique property of suppressing GABA release from inhibitory interneurons, although the exact mechanism for the latter effect is controversial (136,138). The combination of postsynaptic excitation and suppression of inhibition endow kainate receptors with a unique potential to induce epileptic activity. In fact, there is a variety of circumstantial evidence suggesting that kainate receptors may play a role in seizures and epileptogenesis. However, the potential of kainate receptors as antiepileptic drug targets has not been fully explored.

Metabotropic Glutamate Receptors

In addition to its actions on ionotropic NMDA, AMPA, and kainate receptors, synaptically released glutamate also can interact with G-protein-coupled metabotropic glutamate receptors (139). Eight metabotropic glutamate receptors (mGluRs) have been identified to date that are widely distributed in brain. Based on sequence homology, these eight mGluRs are classified into three groups. Group I mGluRs (mGluR1, mGluR5) are positively coupled to phospholipase C and induce hydrolysis of inositol phosphate (PI) and the mobilization of intracellular calcium. Group II mGluRs (mGluR2, mGluR3) and group III mGluRs (mGluR4, mGluR6, mGluR7, mGluR8) are negatively coupled to adenylate cyclase and inhibit the production of cyclic adenosine monophosphate. mGluRs mediate a wide diversity of effects on ion channels, including inhibition of voltage-gated potassium and calcium and nonspecific cation channels (140). In addition, certain group II and III mGluRs, including mGluR2, mGluR4, and mGluR7, are located presynaptically and mediate inhibition of excitatory transmitter release. These diverse actions confer mGluR agonists and antagonists with the capacity both to evoke and inhibit seizures. Indeed, nonselective mGluR agonists and antagonists may have proconvulsant as well as anticonvulsant actions. However, the recent development of selective agents has allowed certain mGluRs to be identified as appropriate anticonvulsant drug targets. Activation of group I mGluRs elicits oscillatory and epileptiform activity *in vitro* (141), and seizure discharges and epileptogenesis in experimental models (142) (as do agonists of other receptors coupled to PI hydrolysis, such as muscarinic cholinergic receptors). Conversely, anticonvulsant effects are often obtained with group II and III mGluR activation (142–144). In contrast to the seizure-inducing properties of group I agonists, group I antagonists, including those that are selective for selective mGluR1 (144,145) and mGluR5 (146), have shown anticonvulsant activity in several seizure models. Among agents targeting mGluRs, group I antagonists seem to have the most clinical potential.

PRESYNAPTIC ACTIONS OF ANTIEPILEPTIC DRUGS

Although blockade of postsynaptic glutamate receptors is not a major factor in the actions of most marketed antiepileptic drugs, effects on other targets—most notably voltage-dependent sodium channels—may indirectly affect glutamate release, thus having the net effect of reducing glutamate-mediated excitatory neurotransmission. In fact, the ultimate way in which sodium channel blocking anticonvulsants protect against seizures may be to a large extent through a reduction in evoked glutamate release. In addition, effects on presynaptic calcium channels and release-regulating receptors such as GABA_B receptors could play a

role in the actions of some antiepileptic drugs including, for example, gabapentin (see earlier).

Synaptic glutamate release occurs when presynaptic nerve terminals are invaded by sodium-dependent action potentials. The subsequent activation of high-voltage-activated (N-, P/Q-type) calcium channels in the axon terminal allows calcium entry that evokes the exocytosis of synaptic vesicles containing glutamate. Studies in brain slices and synaptosomes have indicated that sodium channel blocking anticonvulsants, including phenytoin, lamotrigine, carbamazepine, riluzole, and felbamate, can inhibit glutamate release by virtue of their effects on sodium channels (147–149). Although many of these drugs also can block calcium channels (and as a result inhibit release in an inde-

pendent fashion through this mechanism), effects on these channels typically occur at supratherapeutic concentrations, although for some drugs, such as lamotrigine, the interaction with calcium channels may be clinically significant (150–152). Electrophysiologic recordings of synaptic responses demonstrate that sodium and calcium channel blocking anticonvulsants inhibit action potential-dependent synaptic events without affecting action potential-independent (“miniature”) synaptic events (Figure 1.10). The latter observation reinforces the concept that these drugs do not block postsynaptic receptors and do not interfere directly with the release machinery, supporting the view that the ultimate way in which they protect against seizures is by indirectly suppressing glutamate release. In

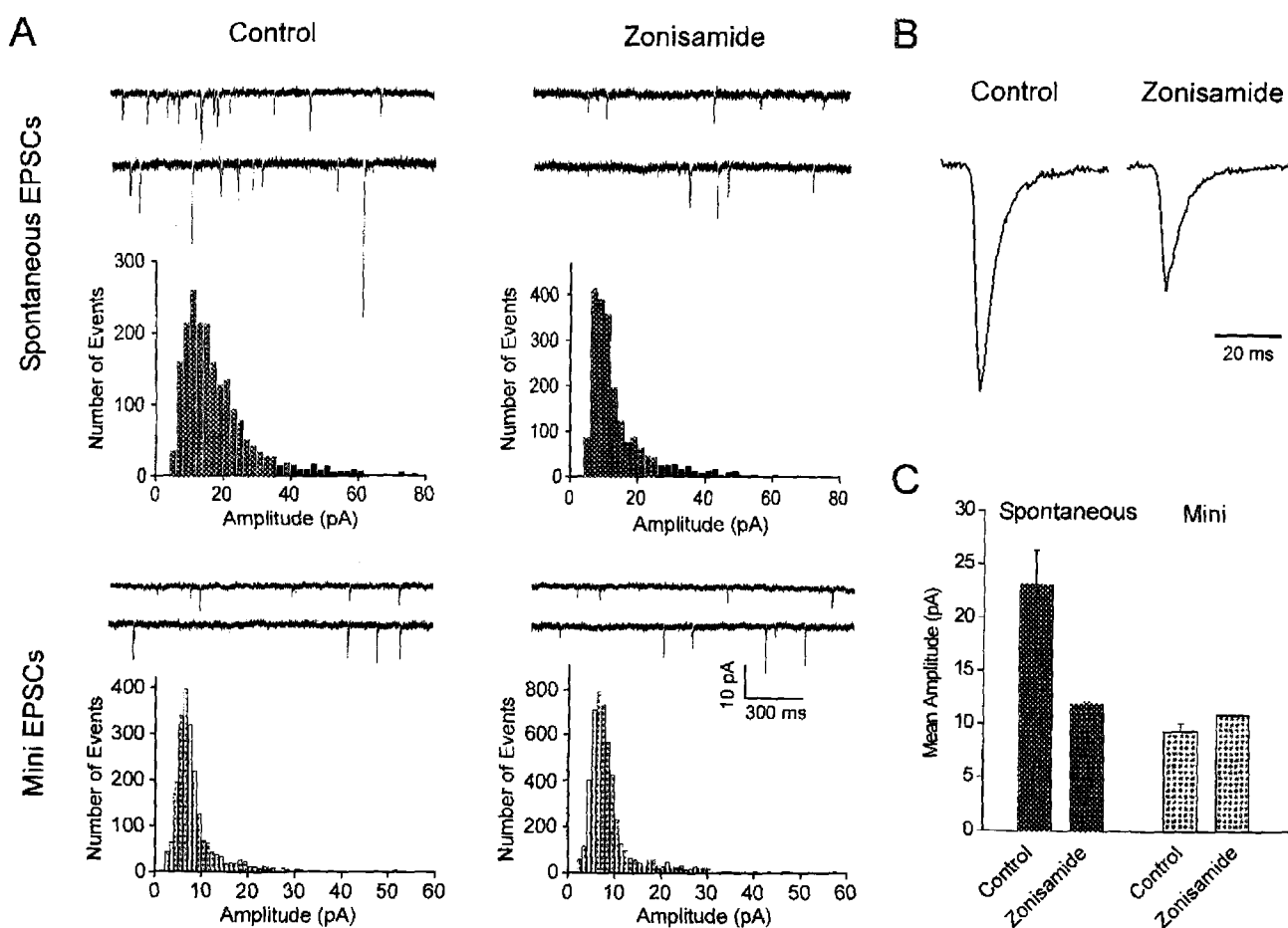


FIGURE 1.10. Sodium channel blocking anticonvulsants suppress action potential-evoked synaptic glutamate release from excitatory terminals. In this series of experiments with zonisamide, whole-cell recordings were made from CA1 hippocampal neurons in brain slices under conditions where GABA_A and NMDA receptors were blocked to isolate fast AMPA receptor-mediated synaptic responses. **A: Top;** the amplitudes of spontaneous action potential-dependent synaptic currents (EPSCs) is reduced in the presence of 20 μ mol/L zonisamide. **Bottom:** Miniature (non-action-potential-dependent) EPSCs recorded in the presence of 1 μ mol/L tetrodotoxin (to block sodium-dependent action potentials) are unaffected by zonisamide, indicating that the anticonvulsant has a presynaptic action (i.e., affects action potential-evoked glutamate release). **B:** Schematic representation illustrating that zonisamide depresses the mean amplitude of action potential-evoked EPSCs, but does not affect their time course. **C:** Comparison of the effects of zonisamide on the mean amplitudes of spontaneous and miniature EPSCs. Holding potential, -70 mV. (Zhu WJ, Rogawski MA, unpublished.)

fact, such drugs may affect GABA release only at relatively higher concentrations as a result of differences in excitation-contraction coupling in glutamatergic and GABAergic neurons (148). However, not all anticonvulsants that inhibit glutamate release do so exclusively through effects on sodium and calcium channels. For example, losigamone may inhibit glutamate release by a mechanism that does not involve sodium channels (153).

ANTIEPILEPTOGENESIS

Currently, antiepileptic drugs are used to reduce or prevent the occurrence of epileptic seizures, and therefore represent symptomatic therapies that do not address the natural course of epilepsy and do not provide a cure. There is no doubt that a preferred approach to therapy would be to prevent the development of epilepsy in those at risk (e.g., in individuals with genetic epilepsies or those with a history of head trauma) or to eliminate seizure susceptibility in those with established epilepsy. In addition, it would be desirable to slow or halt the decline in cognitive functions that many clinicians believe occurs in chronic epilepsy (154). The advent of the kindling model has allowed drugs to be examined for their capacity to interfere with the progressive development of seizure susceptibility (epileptogenesis). In the kindling model, an experimental animal (usually a rodent) subjected to daily, brief deep brain stimulation (usually in the amygdala) progressively develops (usually over the course of 1 week to 10 days) an increased tendency to exhibit a behavioral limbic seizure in response to the stimulation (155). Test agents can be examined for their ability to prevent seizures in fully kindled animals (anticonvulsant action) and also to prevent the development of the enhanced seizure susceptibility (antiepileptogenic action). Kindling also can be induced noninvasively by corneal stimulation (156) or with chemoconvulsants such as pentylenetetrazol (157) and muscarinic cholinergic agonists (158), but the kindled state may not be as persistent and there are important differences in drug sensitivity from the traditional kindling model (159).

NMDA receptor antagonists are the prototypic antiepileptogenic agents. When administered before each kindling stimulation, NMDA antagonists prevent the development of kindling but have little or no effect on the stimulation-evoked afterdischarge (i.e., they do not inhibit the neuronal activation induced by the stimulus), and they also have little or no effect on the expression of kindled seizures in animals that have been kindled in the absence of drug treatment (127,160). Many conventional antiepileptic agents, including phenytoin and carbamazepine, do not prevent epileptogenesis in this model and have only weak activity in protecting against kindled seizures (161). However, some antiepileptic drugs do have antiepileptogenic activity in the kindling model, including valproate (161)

and newer drugs such as levetiracetam (162). In addition, drugs that act primarily on GABA systems, notably tiagabine (163) and vigabatrin (164), as well as phenobarbital (161) and benzodiazepines (165), have antiepileptogenic activity. As yet, the clinical relevance of these observations is uncertain.

MOLECULAR MECHANISMS AND CLINICAL EFFICACY

Pharmacologic studies have demonstrated a wide diversity of molecular targets and mechanisms for antiepileptic drugs. Nevertheless, all antiepileptic drugs appear ultimately to act on ion channel systems, although in some cases the effect is mediated indirectly. The diversity of ion channel targets is remarkable. Structurally novel anticonvulsant compounds—even those that appear similar to older drugs by virtue of their spectrum of actions in animal seizure models—often are found to act on distinct ion channel targets or by new mechanisms. Nonetheless, it is possible to categorize some currently available drugs by mechanism into several broad groups, each with a common spectrum of clinical activities (Table 1.1). Anticonvulsants that act mainly as use- and voltage-dependent *sodium channel blockers*, including phenytoin, carbamazepine, oxcarbazepine, and lamotrigine, are effective in the control of partial and generalized tonic-clonic seizures and are ineffective in the treatment of generalized absence seizures. Agents that *potentiate GABAergic inhibition* by enhancing the synaptic availability of GABA, such as vigabatrin and tiagabine, are effective in partial seizures and may worsen absence seizures. Similarly, barbiturates such as phenobarbital, which augment the function of GABA_A receptors and have additional effects on calcium and other ion channels, are effective in the control of many seizure types but are ineffective in control of absence seizures. In contrast, benzodiazepines, such as diazepam, lorazepam, and clonazepam, which enhance only a subset of GABA_A receptors, are broad-spectrum agents, effective in the treatment of partial, generalized tonic-clonic, generalized absence seizures, and also myoclonic seizures. The specific antiabsence agent ethosuximide seems to act by affecting T-type calcium channels, and possibly also persistent sodium currents. The mechanisms underlying the antiabsence actions of other anticonvulsants, including valproate and lamotrigine, are obscure. Valproate, gabapentin, felbamate, topiramate, zonisamide, and levetiracetam appear to have novel mechanisms (or combinations of mechanisms) of action, possibly affecting calcium channels and glutamate receptors as well as conventional targets, including sodium channels and GABA receptor systems. All of these drugs are effective in the treatment of partial seizures. Topiramate and felbamate probably also are effective in the treatment of absence seizures and other generalized seizure disorders, and are

TABLE 1.1. MOLECULAR TARGETS AND CLINICAL EFFICACY OF ANTIEPILEPTIC DRUGS

Drug	Na ⁺ Channel	Ca ²⁺ Channel	GABA _A Receptor	GABA Transaminase	GABA Transporter	GABA _B Receptor	NMDA Receptor	Clinical Efficacy
Phenytoin	■							Partial, GTC
Carbamazepine	■							Partial, GTC
Oxcarbazepine	■							Partial, GTC
Lamotrigine	■	■						Partial, GTC, absence ^b
Zonisamide ^a	■	■						Partial, GTC, myoclonic ^b
Ethosuximide	■	■						Absence
Phenobarbital		■	■					Partial
Benzodiazepines			■					Broad spectrum
Vigabatrin				■				Partial ^b
Tiagabine					■			Partial
Gabapentin		■				■		Partial
Felbamate	■		■				■	Broad spectrum
Topiramate ^a	■		■					Broad spectrum ^b

GABA, γ -aminobutyric acid; NMDA, *N*-methyl-D-aspartate; I_{NaT} , fast sodium current; I_{NaP} , persistent sodium current; HVA, high-voltage activated; GTC, generalized tonic-clonic seizures.

^aZonisamide and topiramate are weak carbonic anhydrase inhibitors.

^bVigabatrin, lamotrigine, zonisamide, and topiramate may be useful in treating infantile spasms.

therefore broad-spectrum agents. Such agents may be useful, along with broad-spectrum agents like valproate and lamotrigine, in the treatment of mixed seizure syndromes, such as the Lennox-Gastaut syndrome.

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